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EFFECT OF PRE-SOWING SEED TREATMENT WITH SILICA AND FUNGICIDE ON GROWTH, PHOTOSYNTHETIC PIGMENTS AND OXIDATIVE STRESS PARAMETERS OF MUNG BEAN *(VIGNA RADIATA)*

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ABSTRACT

In the present scenario, farmers deal with increasingly volatile weather and their productivity and quality are affected by it.With the growth of new techniques in farming,a large number of methods are there to solve the above problem but the outcomes are not always positive because each field is having different conditions. Seed pretreatment is one of the good findings for them. The present study was carried out to investigate the efficacy of soluble silica and fungicide in the pretreatment of seed before sowing.A highly significant increase inheight of plants, photosynthetic pigments and a highly significant decrease in duration of germination, MDA content and peroxidase activitywere observed in the leaves of mung bean seedlings when seeds were pretreatedwith silica powder.Although a combination of silica powder pretreatment and foliar spray of soluble silica resulted in better growth and reduced oxidative stress. Insignificant changes in studied parameters were observed with fungicide pretreatment. However, liquid soluble silica application through foliar spray along with fungicide pretreatment showed significant changes in improving growth and reducing oxidative stress. It can be concluded from the present study that pretreatment of mung bean seeds with solublesilica powder resulted in enhanced growth and photosynthetic pigments concentration along with the reduction in oxidative stress. Foliar spray of soluble silica applied after the emergence of seedlings gives more pronounced results.

KEYWORD: Agribooster®, Germination, Malondialdehyde, Peroxidase Activity & Soluble Silica Powder

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5555Original Article

1. INTRODUCTION

Germination and emergence of seedlings are the deciding processes for plant growth. Any abnormalities during the emergence of the seedling can restrain the yield and production of a crop (Singh et.al, 2015). Owing to ever-changing climatic and soil conditions, the yield of crops reduces as they are important factors for determining crop production (Mwale et.al. 2003). Several studies have been carried out for reducing time after sowing to the emergence of a seedling. The seed priming technique is one of the important developmental tools related to this field (Heydecker 1973). Seed priming techniques include different treatments that have an impact on metabolic,biochemical and enzymatic activities of seed, thus preparing it to better play their biological functions, such as early germination and seedling establishment (Demir &Mavi 2004).

Seed pretreatment is the process in which some physical, chemical and biological agents are applied before planting to provide effective protection against many seeds and soil-borne plant pathogens (Forsberg *et al.*, 2003). It can be a boon for the farmers having poor resources as it is easy to use and is also economical (Harries 1996).

Different insecticides and fungicides are often used for seed pretreatment to reduce damage caused by many pathogens (Lakhani).Seed pretreatment with different agents that are more environmental and crop friendly compared to commercial treatment in improving the early stages of seedling growth is in great demand (Sehwinn et al;1994). In the present scenario, seed pretreatment has a significant economic impact on markets as it is cost-effective in enhancing productivity (Research and Market, 2013).

Over the last few decades, several authors have observed the significant role of silicon (Si) to induce tolerance against biotic and abiotic stresses, including salinity, high temperature, chilling, drought, etc. It is accelerating seed germination and enhances plant growth and crop yield. It acts as a plant protectant and biostimulant under a range of stress conditions(Zhu& Gong 2014). It also improves the water status and water use efficiency of plants and reduces lipid peroxidation under drought stress (Hasanuzzaman et al; 2018, Liu et al;2019). It could regulate osmolyte accumulation and readjust osmotic potential under water-deficit conditions. It redirects the primary metabolism by acting as a signaling factor under unstressed conditions (Pang et al., 2019; Mohanty et al., 2020). Si is an imperative non-essential plant nutrient that can boost plant resistance against abiotic stresses (Ahmed et al., 2011).

Mung bean (Vigna radiata L. Wilkzek) is a pulse crop with a short duration (70-90 days) and high nutritive value. The seeds contain 22-28% protein, 60-65% carbohydrates, 1-1.5% fat, 3.5-4.5% fibers and 4.5-5.5% ash. **(**Mohamed and Karamany 2005). It is a cheap protein source for direct human consumption (Mubarak 2005).

Keeping the above facts in mind, the present experiment was designed to evaluate the potential of silica as a seed pretreatment agent to improve seed emergence, photosynthetic pigments and reduce oxidative stress parameters of mung bean seedlings.

2. MATERIALS AND METHODS

Mung bean (*Vigna radiata*) seeds MI731-3 variety was purchased from Indian Agriculture Research Institute (IARI) Indore, Madhya Pradesh. Fungicide (Vitavax) @ 2 gm/kg and silica in powdered form (Agribooster® Soluble Silicon Powder) @ 5 gm/kg were used as priming agents. For seedbed preparation and better germination of the crop, the soil was cultivated 2 times with a tractor-mounted cultivator followed by planking each time. Pre-sowing irrigation was applied. Fungicide and soluble silica powderwere mixed with seeds separately and kept for 15 minutes After pretreatment seeds were sowed in a block of area 2m × 2m(4 sq.m) in the field located in the village Loharpipliya, District Dewas. A randomized block design was used and each block represented a single treatment. Foliar spray of liquid silica (10ml/L)in the form of potassium silicate under the trade name Agribooster®was applied twice at the interval of 15 days after sowing.

Following treatments were designed for the study:-

1	T1	Without any pretreatment
2	T2	Fungicide pretreated
3	T3	Fungicide pretreated + Foliar spray of Liquid silica (10ml/L)
4	T4	Soluble silica Powdered pretreated
5	T5	Soluble silica Powdered pretreated + Foliar spray of Liquid silica (10ml/L)

Vegetative, Biochemical and oxidative stress parameters were estimated in leaves of *Vigna radiata* at an interval of 15 days after each treatment. After 15 days of sowing only samples from T1, T2 and T4 were collected.

2.1 Vegetative Parameters Studied in *Vigna radiata*

- **Duration of Germination:** It was noted onthe day when seedlings emerge above the ground.
- **Height of plant:** It was recorded using a standard centimeter scale.

2.2 Biochemical and Oxidative stress parameters studied in leaves of *Vigna radiata* seedlings

- **Chlorophyll and Carotenoid content:** Fresh leaf samples were extracted with 80% acetone. For spectrophotometric determination of chlorophyll a, chlorophyll b and carotenoid contents, the absorbance of the extracts were measured at 645 and 663 nm and 470nmfollowing the method by Lichtenthaler and Wellburn 1983 and calculated using the following formula:

  Chlorophyll (a) in (mg/g) = $12.7(A663) - 2.69(A645) \times V/1000 \times W$

  Chlorophyll (b) in (mg/g) = $22.9(A645) - 4.68(A663) \times V/1000 \times W$

  Carotenoid (mg/g) = $[1000(A470)-3.27 \text{ (Chl a)}- 104 \text{ (Chl b)}]/229$

- **Malondialdehyde content:** The level of lipid peroxidation as an indicator of oxidative stress was measured by using malondialdehyde (MDA), a decomposition product of the polyunsaturated fatty acid present in membrane lipids. It was estimated using thiobarbituric acid (TBA) as the reactive material and measuring absorbance spectrophotometrically at 532 nm.

  Using an extinction coefficient of 155 mM-1 cm-1 following the method of Heath and Packer, (1968).

- **Peroxidase activity**: The enzyme activity was assayed using o-dianisidine as hydrogen donor and H_2O_2 as electron acceptor. The rate of formation of yellow orange colored dianisidine dehydrogenation product was used as a measure of peroxidase activity and assayed spectrophotometrically at 430nm in units/min/g according to Summer and Gjessing, (1943).

3. RESULTS

Table 1: Effect of seed Pretreatment on Parameters Collected after 15 days of sowing

Treatment	Height of plant (cm)	Durationof germination (day)	Chlorophyll a(mg/g)	Chlorophyll b(mg/g)	Carotenoid(mg/g)	MDA(nmoles/gm)	Peroxidase (units/min/g)
T1	6.4 ± .1	4.3±.5	.20±.005	.09±.01	7.90±.02	0.06 ± 0	2.80 ± .05
T2	6.43±.05a^{ns} (.46%)	3.3±.5a^{ns} (23.25%)	.21±.02a^{ns} (5%)	.13±.02a^{ns} (44.44%)	7.96±.45a^{ns} (.75%)	0.07±.01a^{ns} (16.66%)	0.93±.04a*** (-84%)
T4	8.3±.1a**b*(29.68%)	2± 0a**b** (-53.48%)	.22 ± 0a** b^{ns} (10%)	.15±.01a** b^{ns} (66.66%)	8.32±.005a**b** (92.30%)	0.04±.005a*** b** (33.33%)	0.81±.02a*** b* (-86.15%)
* Indicates p value < 0.05 and is significant.** indicates p value < 0.01 and is highlysignificant.*** indicates p value < 0.001 and is extremely significant.a indicates comparison to T1. b indicates comparison to T2.Parenthesis indicates percent increase or decrease							

As shown in table 1, the height of the plant and content of photosynthetic pigments as observed after 15 days of pretreatment showed an insignificant change in fungicide pretreatment. Silica powder pretreatment resulted in a highly significant increase in height of mung bean seedlings and photosynthetic pigments concentration. With the application of silica powder during pretreatment highly significant decrease was resulted in MDA and peroxidase activity as compared to control and fungicide pretreatment. No significant change was observed in the duration of germination on pretreatment with fungicide but a highly exceptional decrease was found in silica pretreatment.

Table 2: Effect of Pretreatment along with Foliar Spray of Soluble Silica on Plant height

Treatment	Height of Plant (cm)	
	After 15 days of first foliar spray	After15 days of second foliar spray
T1	11.6 ± .65	21.11± .32
T2	12.06 ± .81a^{ns}(3.95%)	23.43 ± 1.28a^{ns}(10.99%)
T3	15.23 ± .56 a** b**(31.29%)	25.86 ± .81a***b*(22.50%)
T4	16.17 ± .35a*** b**(39.39%)	26.93 ± .51a***b**(27.56%)
T5	19.46 ± 1.05a*** b**(67.75%)	27.33 ± 1.15a***b**(29.46%)

* Indicates p value < 0.05 and is significant.** indicates p value < 0.01 and is highly significant.*** indicates p value < 0.001 and is extremely significant.a indicates comparison to T1. b indicates comparison to T2.Parenthesis indicatespercent increase or decrease

As shown in table 2, the height of theplant as observed after 15 days of first and second foliar spray showed no significant change in T2(pretreated with fungicide). However, the application of soluble silica through the foliar spray in fungicide pretreated seedlings (T3) showed a significant increment in height of the plant. Silica powder pretreatment(T4) alone and along withfoliar spray of silica also resulted in a remarkable increase in plant height as compared to control (T1) and only fungicide pretreatment (T2).

Table 3: Effect of Pretreatment along with Foliar spray ofSoluble silica on Photosynthetic Parameters

Treatment	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Carotenoid (mg/g)	
	After 15 days of first foliar spray	After15 days of second foliar spray	After 15 days of first foliar spray	After15 days of second foliar spray	After 15 days of first foliar spray	After15 days of second foliar spray
T1	0.22±.005	0.15± .015	0.13±.005	0.07± .005	7.46±.35	5.53±.28
T2	0.24±.01 (9.09%) a^{ns}	0.17±.0 (13.33%) a^{ns}	0.15±.01 (15.38%) a^{ns}	.072±.005 (2.8%) a^{ns}	8.26±.35 (10.72%) a^{*}	5.33±.13(- 3.61%) a^{ns}
T3	0.26±.02 (18.18%) a*** b*	0.18± .01 (20%) a* b^{ns}	0.21±.01 (61.53%) a** b**	0.08 ± 0 (14.28%) a** b*	8.15± .04 (9.24%) a^{**} b^{ns}	6.25±.03 (13.01%) a** b**
T4	0.26±.01 (18.18%) a*** b**	0.19± .0 (26.66%) a** b**	0.25±.005 (92.30%) a** b**	0.13±.02 (85.71%) a** b**	8.26± .01 (10.72%) a** b^{ns}	6.31± 04 (14.10%) a*** b***
T5	0.27±.0 (5%) a*** b**	0.20± .01 (33.33%) a*** b**	0.31±.005 (138.4%) a***b***	0.16±.005 (128.5%) a***b**	9.75± .10 (30.69%) a*** b^{*}	6.77± .07 (22.42%) a***b***

* Indicates p value < 0.05 and is significant.** indicates p value < 0.01 and is highly significant.*** indicates p value < 0.001 and is extremely significant.a indicates comparison to T1. b indicates comparison to T2.Parenthesis indicates percentincrease or decrease

Photosynthetic pigments as observed after foliar spray with soluble silica at the interval of 15 days showed insignificant change in the leaves of T2 (pretreated with fungicide only). As shown in table 3, a highly significant increase in chlorophyll a, chlorophyll b and carotenoid concentration was found in T3 (pretreated with fungicide along with foliar spray), T4 (Pretreated with solublesilica powder alone) and T5 (pretreated with solublesilica powder along with foliar spray).

Table 4:Effect of seed Pretreatment along with Foliar Spray ofsoluble silica on Oxidative Stress Parameters

Treatment	MDA (nmoles/gm)		Peroxidase (units/min/g)	
	After 15 days of first foliar spray	After 15 days of second foliar spray	After 15 days of first foliar spray	After 15 days of second foliar spray
T1	.05 ± .02	0.03 ± .006	5.45 ± .27	6.22 ± .06
T2	.03 ± .02 a^{ns} (- 40%)	0.019± .01 a^{ns} (-36%)	2.61 ±.01 a*** (-76.07%)	2.84 ±.23a***(-65.49%)
T3	.018±.004 a* b^{ns} (-64%)	0.012± .004 a^{*} b^{**} (-60%)	2.53 ±.12 a*** b^{**} (-76.81%)	2.48 ± .026 a*** b^{**} (-69.86%)
T4	.017± .002 a** b^{ns} (-66%)	0.013± .004 a** b^{ns} (-56%)	1.92 ± .04 a*** b^{**} (-82.40%)	1.54 ±.07 a*** b^{**} (-81.28%)
T5	.009± .0005 a*** b^{*} (-82%)	0.007± .001 a^{**} b^{***} (-76%)	1.89 ± .07 a*** b^{**} (-82.62%)	1.29 ± .05 a*** b^{**} (-84.32%)

* Indicates p value < 0.05 and is significant.** indicates p value < 0.01 and is highly significant.*** indicates p value < 0.001 and is extremely significant.a indicates comparison to T1. b indicates comparison to T2.Parenthesis indicates percentincrease or decrease

As shown in table 4, application of silica through the foliar spray twice at the interval of 15 days showed an insignificant reduction in MDA content and activity of peroxidase enzyme in the leaves of mung bean seedlings obtained from T2 (fungicide pretreated) and a highly significant decrease was observed in T3 (pretreated with fungicide along with foliar spray), T4 (Pretreated with solublesilica powder alone) and in T5 (pretreated with solublesilica powder along with foliar spray).

Figure 1: Effect of Pretreatment with Fungicide and Silica along with Foliar Spray of Silica on Plant height og Mung Bean.

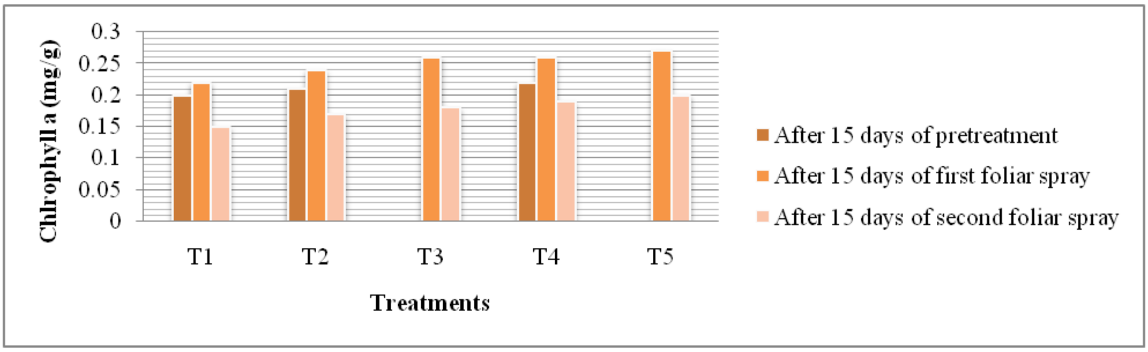

Figure 2: Effect of Pretreatment with Fungicide and Silica along with Foliar Spray of Silica on Chlorophyll 'a' Content in Leaves of Mung Bean.

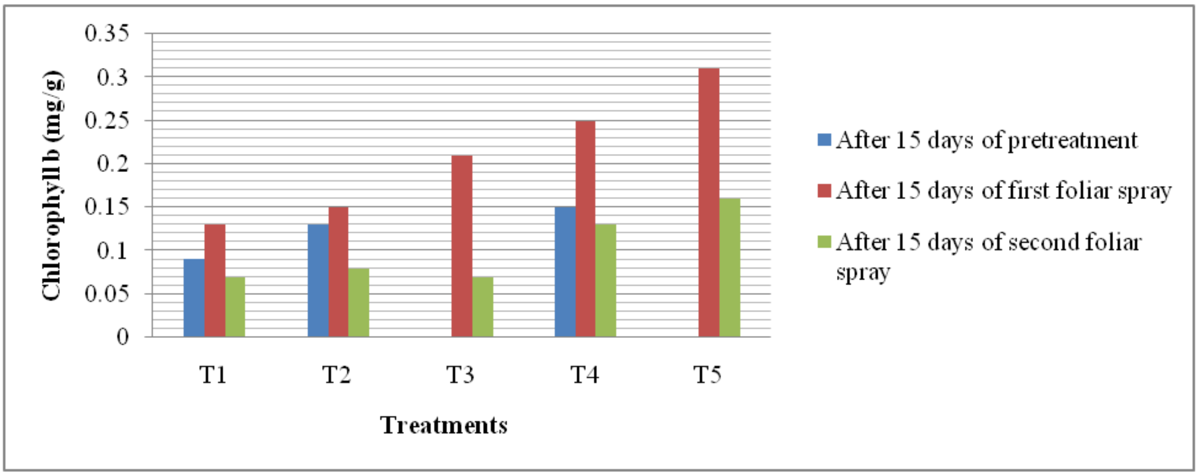

Figure 3: Effect of Pretreatment with Fungicide and silica along with Foliar Spray of Silica on Chlorophyll 'b' Content in Leaves of Mung Bean.

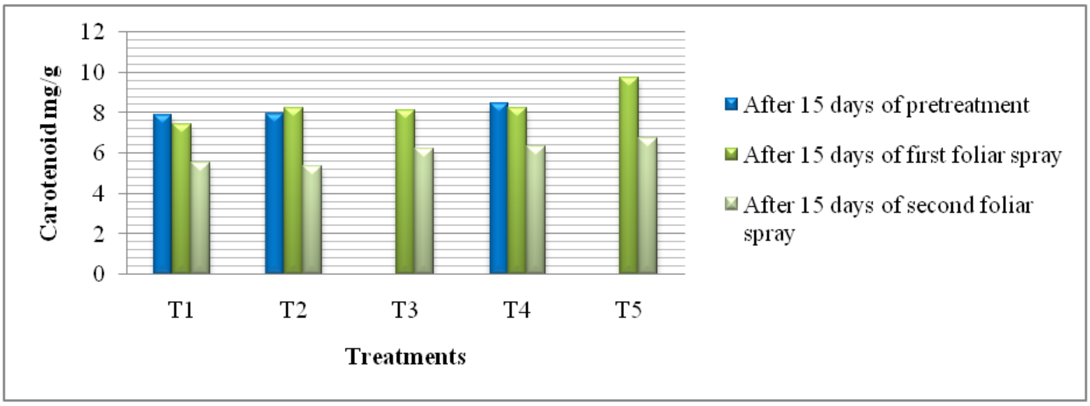

Figure 4: Effect of Pretreatment with Fungicide and Silica along with Foliar Spray of Silica on Carotenoid Content in Leaves of Mung Bean.

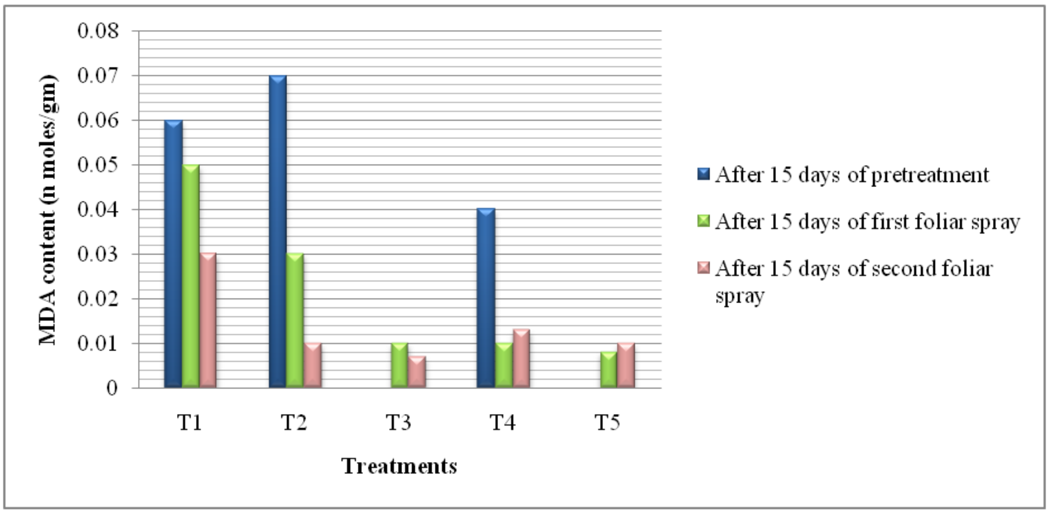

Figure 5: Effect of Pretreatment with Fungicide and Silica along with Foliar Spray of Silica on MDA Content in Leaves of Mung Bean.

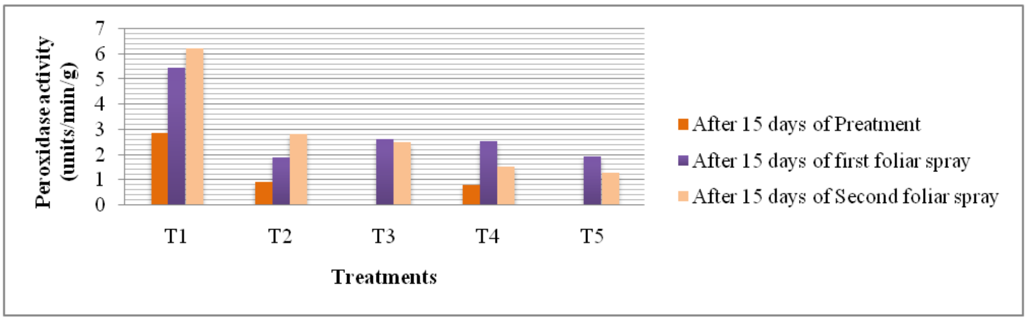

Figure 6: Effect of Pretreatment with Fungicide and (Soluble) Silica along with Foliar Spray of Silica on Peroxidase Activity in Leaves of Mung Bean.

4. DISCUSSIONS

4.1 Vegetative Parameters

Proper germination is very essential for the continued growth and yield of the crop. From the result obtained, solublesilica supplementation in pretreatment significantly enhanced the germination of mung beans. Siddiqui, etal., 2014 &Foltete et al., 2011 also reported increased seed germination, mean germination time, germination index and seed vigor index in tomato with the application of silicon. In water deficit conditions increased germination of seed was observed with the supplementation of calcium silicate (Zarger et al. 2013). Reasons behind this elevation as reported by (Lei et al 2008) is improved ability of seed for absorbing and utilizing water and fertilizer in presence of silicon which promotes seed antioxidant system, reduces stress by reducing superoxide radicals and increasing activity of some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase in spinach.

The application of soluble silica powder as a pretreatment agent before sowing significantly increased the height of the plants of *Vigna radiata*in comparison to control (Figure 1). Likewise, THE results shownby Janmohammadi et al., 2015 on the pre-hydration treatment of seed with the colloid solution of silica nanoparticles before germination significantly improvedheight parameters insunflower (*Helianthus annuus L.*).

4.2 Photosynthetic Pigments

The role of chlorophyll and carotenoids in photosynthesis stabilization of membranes and energy transformation has been studied widely (Zang 2003,Jiang et al., 2017). The chlorophyll production is mainly dependent on the penetration of sunlight and it is the main component for photosynthesis (Srichaikul et al; 2011). It is influenced by different stress factors.Silicon provides rigidity and strength, enables erectness of leaves, which results in enhanced light absorption and improved photosynthesis, photo assimilation, growth and final yield of the plant (Saud et al. 2014).

Reduced chlorophyll content of tomato leaves due to drought stress was significantly increased by the addition of silicon which resulted in enhanced activity (Song et al., 2014). Underwater stress, silicon-mediated improvement in chlorophyll content may be due to reduced production of ROS in presence of silicon leading to improved antioxidant defense (Shi et al.2016).Results similar to the present study were observed in coriander leaves supplemented with silicon under water-deficient stress. Silicon supplementation led to the elevated growth rate of photosynthesis, yield and water using capacity of plants grown under reduced water availability(Gao et al., 2005). Silicon is likewise recognized to improve the resistance of plants towards drought through enhancing photosynthetic performance, maintaining plant water equilibrium and straightness of leaves (Yin et al.2013). Silicon increased expression of genes which encodes for enzymes involved in the synthesis of precursors for chlorophyll (protoporphyrin IX, Mg-protoporphyrin IX and protochlorophyllide) under nitrate stress may be the reason for the improvement in chlorophyll content is the presence of silica. (Candan&Tarhan2003; Taiz & Zeiger 2009). Our study results also showed improvement in content of chlorophyll a, chlorophyll b and carotenoid in the leaves of mung seedlings pretreated with soluble silica powderbefore sowing along with foliar spray of soluble silica twice at the interval of 15 days after sowing (Figure 2, 3,4).

4.3 Oxidative Stress

In open field everchanging climatic conditions exposes plants to stress factors which lead to the production of many ROS in the course of photosynthesis and respiration (Milttler 2002, Arbona et al., 2003).For maintenance of homeostasis in plants, two mechanisms for detoxification are observed which include enzymatic and non enzymatic antioxidants(Sytar et al., 2013, Wuet et al., 2017). In stress caused due to salinity, significant reduction in the content of peroxidase, catalase activity was also observed by (Tarabi et al.2015, Kim et al.,2016 and Tripathi et al., 2017). Oxidative stress marker in terms of MDA was reduced and peroxidase activity was increased with pretreatment of mung seeds with soluble silica powder before sowing. Application of soluble silica through foliar spray after sowing further reduced content of MDA and enhanced activity of peroxidase indicating decrement in oxidative stress (Figure 5, 6). Similar results with supplementation of silicon indicating a decrease in MDA content in salt-stressed Barley, maize and grapevine rootstock was reported which maintained integrity of membrane and reduced permeability.

5. CONCLUSIONS

Seed pretreatment with silica is an environmentally safe and new wave that can be easily adopted by resource-poor farmers and benefited the farmers in various ways. It may indeed be considered as a valuable strategy to improve stand establishment under adverse agro-climatic conditions (rainfed, dry farming and dryland farming regions) with enhanced yield, increased tolerance to stress situations, increased resistance against diseases, enhanced crop competitiveness against weeds and increased water use efficiency. The pretreatment with Soluble silica powder was more beneficial in improving vegetative growth and photosynthetic pigment than fungicide. The farmers are advised to use Soluble silica powder for the

pretreatment of seeds as it results in better germination and growth of the seed. Thepretreatment with soluble silica powder along with foliar spray with soluble silica can further enhance quality by enhancing growth and reducing oxidative stress. Thus in addition to silica pretreatment, foliar spray with soluble silica is also suggested to the farmers to increase their productivity.

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